

CHEESE POISONING:
A TOXICOGENIC BACILLUS
ISOLATED FROM CHEESE

BY

WILLIAM LEVIN, M.S.P.H.,

Ann Arbor, Mich.

A dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Public Health in the University
of Michigan, June, 1917.

Reprint from

THE JOURNAL OF
LABORATORY AND CLINICAL MEDICINE

Vol. II, No. 11, August, 1917



ss. Ann Arbor
1917



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41551522_0011

CHEESE POISONING: A TOXICOGENIC BACILLUS ISOLATED FROM CHEESE*

BY WILLIAM LEVIN, M.S.P.H., ANN ARBOR, MICH.

HISTORICAL INTRODUCTION.

CHEESE poisoning, according to Husemann,¹ was already known in North Germany in the sixteenth century. Christison,² in his "Treatise on Poisons," stated: "During the third quarter of the last century (eighteenth) this kind of poisoning was so common that several of the German states investigated the subject, and legislative enactments were passed in consequence. For a long time the prevalent belief was that the cheese acquired an impregnation from copper vessels used in the dairies, and accordingly the Austrian, Wirtemberg, and Ratesberg states prohibited the use of copper for such purposes. This opinion was proved by chemical analysis to be untenable." Morgagni³ mentions arsenic as the cause of cheese poisoning. Later investigators ascribed the poisonous properties to lead, zinc, and mercury.

Early in the nineteenth century the experimental method of inquiry was begun; and although it was not until the latter half of the century that the cause was definitely established, the work done in the interim was of value from the negative point of view. Hunnefeld,⁴ in 1827, analyzed poisonous cheese and experimented with extracts upon the lower animals. He considered the active poisoning agents to be sebacic and caseic acids. "About the same time, Serturner, making analyses of poisonous cheese for Westrumb, also traced the poisonous principles, as he supposed, to these fatty acids."⁵ This view was adhered to by succeeding investigators until 1852, when Schlossberger proved conclusively that the fatty acids, in a pure state, were devoid of poisonous properties.

In the years 1883 and 1884 there were reported to the Michigan State Board of Health about three hundred cases of cheese poisoning. Vaughan,⁶ working on the cheese sent to him for analysis, was able to isolate a ptomaine, called by him tyrotoxin, which he considered the active agent in the poisonous cheese. His work was corroborated by Wallace⁷ in 1887, by Wesener and Rossman⁸ in 1898, and by Newman⁹ in 1902. Lepierre,¹⁰ in 1894, isolated a base having the formula $C_{16}H_{24}N_2O_4$ from poisonous cheese. Dokkum,¹¹ in 1895, isolated a base, a currare-like poison, from putrefactive cheese, which he called tyroxin. Recently (1910) Spica¹² obtained a toxic extract, of unknown chemical composition, from poisonous cheese.

From the work of these men it is evident that they considered cheese poisoning an intoxication. In classifying the various bases obtained from poisonous cheese with the ptomaines, they moreover admitted the poisoning to be primarily due to bacterial activity. For a ptomaine, according to Vaughan and Novy,¹³ "may be defined as an organic chemical compound, basic in character, and formed by the action of bacteria on nitrogenous matter." The question

*A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Public Health in the University of Michigan.

then arose: "Can pathogenic or toxicogenic bacteria be shown to be present in poisonous cheese?"

Bacterial analysis of poisonous cheese had already been undertaken by Sternberg, at the request of the Michigan State Board of Health in 1884. In his report¹⁴ to that body he stated that he found "micrococci in the fluid of the cavities of the cheese." Subcutaneous injections of the pure cultures into rabbits were without effect. He concluded: "It seems not improbable that the poisonous principle is a ptomaine developed in the cheese as a result of the vital activity of the above mentioned micrococcus, or of some other microorganism which had preceded it, and had perhaps been killed by its own poisonous products."

Novy,¹⁵ in 1890, isolated an organism from poisonous cheese which, when inoculated in milk and kept in the incubator, was fatal to cats. Vaughan and Perkins,¹⁶ in 1895, isolated a toxicogenic bacillus from cheese which had proved poisonous to twelve persons. Their bacillus belonged to the colon group. Holst,¹⁷ a year later, described a colon-like bacillus which he had obtained from poisonous cheese. Fonteyne,¹⁸ in 1908, obtained a Gram-positive, pathogenic bacillus from a sample of cheese which had caused a gastrointestinal epidemic at Meirelbeke among forty persons. Kühn,¹⁹ in 1913, working with a cheese that had caused poisoning to about fourteen persons, isolated a pathogenic organism which he considered similar to the *Escherich lactis aerogenes* bacillus. In all these cases it was demonstrated by animal experimentation that the organism isolated caused the poisoning. Vaughan and Perkins were able to show that their organism formed a soluble poison, as sterile filtrates of their cultures, when injected intraperitoneally, into guinea pigs, caused death. In the other cases of cheese poisoning from bacterial activity no studies were made, or at least reported, upon the toxicogenic properties of the organism.

An outbreak of cheese poisoning among several members of a family in Kalamazoo, Michigan, offered the opportunity to investigate and study the cause of the poisoning. The results of this investigation are embodied in this report.

HISTORY OF THE CASES.

Mr. and Mrs. B. W. M. and family of four children, of Kalamazoo, Michigan, on June 20, 1916, were taken seriously ill several hours after having eaten supper, which had included American cheese. Dr. W. S. Tomkinson, who was called in, sent a sample of the cheese, of about 100 grams, and a history of the case to the Hygienic Laboratory of the University of Michigan. His report follows:

"Case 1. Mr. B. W. M., osteopath, ate supper, including limburger cheese, about 6 P.M. At 12 P.M. purging; vomiting; face pale, muscles trembly; eyes dilated; pulse 110, irregular. Vomit thin, reddish color with small chunks of cheese and undigested meat. 12:10 A.M. gave tablespoonful magnesium sulphate in $\frac{3}{4}$ ii hot water. Purging profuse. O. K. at 3 A.M. with painful abdomen and hypothorax.

"Case 2. Mrs. B. W. M. Same as Case 1. Pulse 100, irregular; weak. 12 P.M., vomiting and straining, with flushed face. No purging. 12:10 gave magnesium sulphate tablespoonful to $\frac{3}{4}$ ii hot water. Vomited immediately. Repeated dose; vomited in five minutes. Patient grew worse and rolled on floor with stomach cramps. Gave $\frac{3}{4}$ i epsom salts to pint of hot water with immediate results and relief. O. K. 3 A.M.

"Case 3. Oldest boy, age 11 years. Was feeling better when I reached house at 12

midnight after purging and vomiting. Epsom salts cleaned out bowels thoroughly and gave relief.

"Case 4. Girl, age 8 years. At 6 P.M. ate heartily of cheese. 12 P.M., vomiting. Mother gave 2 $\bar{3}$ castor oil. Girl quieted down and went to sleep. At 12:20 I woke her up and found she had a chill. Cold sweat. Pulse slow, irregular. She was weak and trembly. Eyes dilated, face pale, cyanotic. Said she felt all right, but was cold and wanted to be covered up and go to sleep again. Gave $\bar{3}$ ss magnesium sulphate which was thrown out of stomach with such force it struck opposite wall three feet away. Many small chunks of cheese in vomitus. Washed out stomach with warm water which returned tinged red. Repeated epsom salts $\bar{3}$ ss, water $\bar{3}$ ii, which started purging with relief. Patient O. K. and asleep at 2 A.M. Pulse weak, rapid, but regular. Color returned to face.

"Case 5. Boy, age 6 years. Asleep at midnight was awakened and took epsom salts $\bar{3}$ ss, hot water $\bar{3}$ ii, and immediately fell asleep again. Slept 20 minutes, after which purged out thoroughly. Pulse 60, slow, very irregular. Pupils dilated, face cyanotic, cold sweat. Not in as much pain as rest. After purging thoroughly—face flushed—went back to bed feeling comfortable. O. K. 3 A.M.

"Case 6. Baby boy, age 16 months. Ate sparingly. Gave magnesium sulphate $\bar{3}$ ss, hot water $\bar{3}$ ii, and put to bed. Slight cramps; free bowel movement. No vomiting. O. K. 3 A.M.

SYMPTOMS.

The symptoms as given by Tomkinson are those of known cases of cheese poisoning,—a fact that led him to regard his cases as those of tyrotoxismus. As a rule, the symptoms are of a severer character. Early observers^{20, 21, 22} gave these symptoms as characteristic of cheese poisoning: chills, paleness of face, severe vomiting, diarrhea, marked pains in stomach and intestines, trembly limbs, thirst and dizziness. Pollius,²³ Wallace,⁷ and Holst¹⁷ mention vomiting of blood. Fonteyne¹⁸ stated that some of his patients passed bloody stools. At times marked nervous disturbances may occur, as in a case cited by Vaughan,¹⁶ where a patient became violently delirious. In the Michigan epidemic of 1883-1884²⁴ the symptoms produced by poisonous cheese were: dryness of the throat, nausea, vomiting, diarrhea, nervous prostration, headache, and sometimes double vision.

INCUBATION PERIOD.

Significant and important is the interval of time between the eating of the cheese and the appearance of the first symptoms of poisoning,—an interval which must be regarded, from the majority of cases, as an incubation period. This terminology would imply that cheese poisoning should be regarded not as an intoxication but rather as an infection. This is, indeed, the opinion of the present-day investigators.

The incubation period in the cases with which this report is concerned was, it may be remembered, six hours. It would be reasonable to assume that, were we dealing with an intoxication, the symptoms would have appeared much earlier. Instances are cited in the literature^{1, 20, 21} where the interval of time between the eating and the appearance of the symptoms was from one to two hours. Westrumb² mentioned half an hour interval. In the Michigan epidemic of 1883-1884 some persons were taken ill from thirty minutes to four hours after eating. In such cases a preformed poison may be considered, like that of Vaughan's tyrotoxicon. In all cases, however, where bacteria were isolated

from poisonous cheese, the interval was not less than five or six hours,—a period amply sufficient for bacterial activity.

DURATION OF SICKNESS.

Prompt treatment by the attending physician resulted in a rapid recovery by the Kalamazoo patients. The poisoning, however, to which these people were subjected, was not so marked as in many of the cases cited in the literature. To this can be attributed the short duration of their sickness. Instances are not lacking where days and even weeks elapsed before the recovery of the patients. Thus Grimm²⁰ stated the period to be three days; Pollius,²³ eight to twenty-four hours; Zenker,²² twenty-four hours; Wallace,⁷ three days; Ehrhardt,²⁵ "a long convalescence;" Newman,⁹ forty-eight hours; Hughes and Healey,²⁶ four to eight days; Holst,¹⁷ four days to three weeks; and Kühl,¹¹ ten days.

MORTALITY.

Deaths through cheese poisoning make up a very small percentage of the total number of cases. In the Michigan epidemic no fatalities were recorded. Lochte²⁷ tabulated eleven fatal cases from cheese poisoning up to 1903. Berg²⁸ mentions a fatal case occurring in 1908.

POSTMORTEM APPEARANCE.

Not all the fatal cases gave the same postmortem pictures. Hunnemann¹ found the stomach and intestines to be inflamed. Cameron,²⁹ quoting Holm's³⁰ postmortem findings, said "There was extravasated blood in the lungs, which were slightly congested; they decrepitated." The left ventricle of the heart contained liquid blood; the right auricle and two left cavities contained numerous soft and recent clots. The stomach and other abdominal viscera did not present any abnormal appearances which would account for death." Hughes and Healey,²⁶ in their report of an epidemic of gastroenteritis attributed to cheese poisoning (tyrotoxicon having been found in the cheese) at Aldershot, England, in 1899, gave the following postmortem appearances in three fatal cases: "Intense congestion; inflammation and edema of the mucous membrane of the stomach and small intestines, varying in degree and locality, but in one case involving the stomach and the whole of the small intestine. Peyer's patches were practically unaffected, but the solitary glands were congested and prominent. There was no ulceration or visible entozoa and the colon was unaffected. In one case the small intestine contained loose terra-cotta colored fecal material. The liver, which was enlarged (from 70 to 78 ounces) and bile-stained, showed signs of fatty degeneration. The gall bladder was full but not markedly distended. The spleen was normal. The kidneys were enlarged and intensely congested; the capsule was nonadherent. In one case the cortex of both kidneys contained small cysts filled with clear fluid. The skin and other tissues were deeply bile-stained. The lungs were congested posteriorly. The thoracic and abdominal lymphatics were not noticeably enlarged." Berg's case²⁸ presented a picture of cholera nostras. It seems, however, that in many cases marked anatomical changes do not occur.

APPEARANCE OF THE CHEESE.

Tomkinson, the physician attending the M. family, sent a sample of the cheese and also of the meat eaten at that meal. The meat, evidently a pork chop, was found on bacterial examination, to contain only *Bacillus mesentericus vulgatus*, and hence was dismissed from consideration as the source of poisoning. The sample of cheese was of about 100 grams in weight, of the variety known as American (wrongly termed Limburger by Tomkinson), and of normal appearance and odor. This normal appearance corroborates the statements of many investigators that the cheeses causing poisoning were seemingly in excellent condition.

EXPERIMENTAL WORK.

Feeding Experiments.—Experiments were conducted with a view of determining whether poisoning could be induced in laboratory animals by feeding them the suspected cheese.

TABLE I.
FEEDING SUSPECTED CHEESE.

June 24, 1916.	Guinea pig No. 1, 220 gm., fed 10 gm. cheese.	No effects.
June 24, 1916.	Rats No. 1 and No. 2, both fed 5 gm. cheese.	No effects.
June 24, 1916.	Mice No. 1 and No. 2, both fed 5 gm. cheese.	No effects.

None of the animals fed showed the least symptoms of sickness. This was to be expected in view of similar findings by other authors. Nüchel,³¹ in 1836, stated that a dog was unaffected eating cheese that had caused marked poisoning in a man. Pollius²³ claimed to have observed trembling in a mouse fed with poisonous cheese. Holm³⁰ obtained negative results in his feeding experiments. Sternberg¹⁴ reported negative results by feeding rats and dogs. Vaughan said,³² "If two samples, one of good, the other of poisonous cheese, were placed before a dog or cat, the animal would invariably select the good cheese; but if only poisonous cheese was offered, and the animal was hungry, it would partake freely. A cat was kept seven days and furnished only poisonous cheese and water. It ate freely of the cheese and manifested no untoward symptoms. After the seven days the animal was etherized and abdominal section was made. Nothing abnormal could be found." Holst¹⁷ and Kühl¹⁹ also obtained negative results in their feeding experiments.

ALCOHOLIC EXTRACTS OF CHEESE.

After removing aseptically a portion of the suspected cheese for bacterial analysis the remainder, 65 grams, was chopped up finely and digested with 150 c.c. absolute alcohol for three days at room temperature. It was then filtered, the filtrate evaporated to dryness, taken up in 50 c.c. distilled water, heated on a water bath, and filtered into a weighed dish. On evaporation to dryness a residue of 1.634 gm. was obtained. This was then taken up in 50 c.c. distilled water, and portions of 5 and 10 c.c. each were injected, intravenously, into guinea pigs. The animals showed no symptoms of disturbance. The same procedure with the sample of the pork chop also gave negative results. It was concluded, therefore, that no alcohol-soluble substance caused the poisoning, and that the source might possibly be traced if a bacterial analysis were made.

ISOLATION OF THE ORGANISM—TECHNIC.

With a sterilized knife a section of cheese was cut so that all surfaces were free from external contamination. This was then placed in a sterile test glass, physiological salt solution added, the cheese thoroughly macerated with a sterile rod, and the resulting suspension filtered through a sterile cotton filter. 1 c.c. portions of the filtered suspension were then inoculated into glucose gelatin, milk, and bouillon tubes, and the latter two incubated at 37° C. for 24 hours. A layer of sterile oil to about a quarter of an inch in depth was put over the glucose gelatin and these tubes incubated at room temperature. At the end of 24 hours the glucose gelatin showed marked gas formation, the milk was curdled, and the bouillon had a very heavy growth.

From these three different media, after plating on agar and fishing numerous colonies, an organism was isolated which, when grown in milk, proved pathogenic to mice. The same organism was isolated from glucose agar plates, grown anaerobically in Novy jars, in an atmosphere of hydrogen gas.

MORPHOLOGY.

The organism is a facultative anaerobic bacillus, resembling in form and dimensions the *Bacillus colon*. The size of the bacillus varies from coccus-like forms to large rods, depending upon the kind and age of the media in which it is grown. As a rule its length is about three times its width. The organism grows singly and also in the diplo form. Thread formation has been infrequently observed. It does not form spores.

MOTILITY.

Depending upon the media and the conditions of growth the bacillus may show active motility, sluggish motility, or even marked Brownian movement. Table II shows the influence of the kind of media upon the size and motility of the organism.

TABLE II.
THE INFLUENCE OF MEDIA UPON SIZE AND MOTILITY.

AGE	MEDIUM	SIZE	MOTILITY
24 hours	Bouillon	Small rods; several large rods	Actively motile, large rods sluggishly motile
24 hours	Agar	Small rods	Actively motile
72 hours	Agar	Coccus-like bacilli	Motile
24 hours	Lactose broth	Slender rods, many large	Sluggishly motile, a few actively
24 hours	Dextrose broth	Plump rods, some very long	Marked Brownian movement; few slightly motile
24 hours	Saccharose broth	Small rods	Actively motile; many show no motility
24 hours	Milk	Small rods	Actively motile
72 hours	Milk	Small rods	Motile
24 hours	Uschinsky's medium	Fairly long rods	Many actively, some sluggishly motile
24 hours	Bile lactose	Small slender rods	Marked Brownian movement
Peritoneal fluid from guinea pig		Small plump rods	Few actively motile; most marked Brownian movement

STAINING PROPERTIES.

The organism takes all stains readily. With Loeffler's methylene blue the ends of the bacillus stain a dark blue, while the inner portion resembles a vacuole. It is Gram-negative and not acid-fast. Smears made from the organs of an animal killed by the bacillus, when stained according to the Welch method, show the organisms to have capsule-like bodies around them. These, however, could never be verified as capsules.

GROWTH ON GELATIN.

On gelatin plates the colonies are usually of two forms,—oval, characteristic of the deep colonies, and round, with irregular borders, characteristic of the surface colonies. Stabs show filiform growths, the growth being best at top. The gelatin is not liquefied.

GROWTH ON AGAR.

The surface colonies on agar plates are round, with regular borders. Distinct concentric rings from two to three in number can be observed,—the inner ring being darker. The deep colonies are oval. When grown on glucose agar plates, large bubbles of gas, at times approaching 1.5 cm. in diameter, are formed. On agar slants the growth is abundant, filiform, and glistening. An odor is given off strongly resembling that of *B. coli* cultures. No pigment formation has been observed. On Drigalski-Conradi medium it forms pink colonies similar to *B. colon*. On Endo medium the colonies are red with the characteristic metallic caps of colon colonies.

GROWTH ON POTATO.

The growth on potato is noncharacteristic. Depending upon the reaction of the potato the growth may be scanty or abundant—a slightly alkaline reaction favoring the latter. Where abundant, it is of a slimy consistency, raised, and at times spreading. After several days the potato becomes more or less browned.

GROWTH IN NUTRIENT BROTH.

The bacillus grows readily and rapidly in nutrient broth, distinct clouding being visible in as short a period as three hours. A slight ring forms at the surface, but this may, at times, be lacking. No pellicle formation takes place. The clouding is strong at the end of twenty-four hours. On standing several days a flocculent sediment forms, with a slight clarification of the culture. The odor here again resembles that of a *B. colon* culture. In sugar broths growth is abundant, with formation of gas and acids.

GROWTH IN MILK.

When first isolated the bacillus would curdle milk at the end of about four to five days. The curd in that case would be slimy. After passage through many animals (approximately 80), the organism curdled milk in forty-eight hours, and the curd was distinct from the whey. Gas bubbles could be observed at the surface of the culture. The acidity formed varied from 0.2 per cent to 0.3 per cent.

GROWTH ON BLOOD SERUM.

On this medium the organism forms a moderate, slightly glistening, filiform growth.

GROWTH IN USCHINSKY'S SOLUTION.

The solution was similar to that used by Vaughan and Perkins and was made up as follows:

Glycerine	40 parts
NaCl	1 part
CaCl ₂	0.1 part
MgSO ₄	0.4 part
K ₂ HPO ₄	2.5 parts
Ammonium Lactate	6.0 parts
Asparagin	3.4 parts
Water	1000 parts

The bacillus grew copiously in this solution. In Fränkel's modification of Uschinsky's medium no growth was observed at twenty-four hours. A light growth was visible at forty-eight hours, which increased to a heavy growth at seventy-two hours. The laboratory strain of *B. colon*, and a strain isolated from feces also showed no visible growth in this solution at twenty-four hours, while both gave good growths in the Uschinsky medium for the same period. This is interesting in view of the fact that Vaughan and Perkins found their organism would not grow in Uschinsky's medium if the dipotassium hydrogen phosphate were replaced by disodium hydrogen phosphate. The latter salt, as can be seen from the following formula, was used in Fränkel's medium:

Asparagin	4.0 gm.
Ammonium Lactate	6.0 gm.
Na ₂ HPO ₄	2.0 gm.
NaCl	5.0 gm.
Water	1000 c.c.

TEMPERATURE REQUIREMENTS.

The optimum temperature is about 37° C. The organism grows also well at room temperature. At high temperatures, as at 45° to 50° it seems to lose its power of fermenting sugars. It grows scantily below 10° C. Cultures grown at 37° C. appear to be the most toxic.

THERMAL DEATH POINT.

The thermal death point of the organism was determined by both the moist and dry heat methods, using silk thread, according to the technic employed in the Hygienic Laboratory of the University. The bacillus, as can be seen from the following tables, although withstanding moist heat at 50° C. for two hours is readily killed at 60° C. It is practically nonresistant to dry heat.

TABLE III.
THERMAL DEATH POINT. MOIST HEAT.

Time in Minutes	½	1	2	3	5	10	15	30	45	60	120
50° C.							+	+	+	+	+
55° C.							+	-	-	-	-
60° C.		+		-	-	-	-	-	-	-	-
70° C.		-		-	-	-	-				
100° C.	-	-	-	-	-	-	-				

TABLE IV.
THERMAL DEATH POINT. DRY HEAT.

Time in Minutes	5	15	30	45	60
100° C.	+	-	-	-	-
120° C.	-	-	-	-	-
130° C.	-	-	-	-	-
150° C.	-	-	-	-	-

Controls for both moist and dry heat = +.

FERMENTATION.

Various sugars were used to test the fermenting property of the organism and also to differentiate it thereby, if possible, from the colon bacillus. The media used were 2 per cent sugar broths neutral to phenolphthalein. Before inoculation the tubes were allowed to stand at room temperature for 12 hours. Two colon strains were used as comparatives: one, the laboratory strain, and the other, a strain isolated from feces. Loopfuls from 24 hours' cultures of each of the three organisms were inoculated into the fermentation tubes, and these incubated at 37° C. Readings were taken at intervals of 24, 48, and 72 hours.

For convenience the bacillus isolated from the cheese will be termed *Bacillus* 3B.

TABLE V.
FERMENTATION COMPARATIVES. PER CENT GAS.

Hours	B. COLON FECES			B. COLON LAB.			BACILLUS 3B.		
	24	48	72	24	48	72	24	48	72
Xylose	10	25	30	10	25	30	20	30	30
Arabinose	15	25	30	15	30	30	15	25	25
Dextrose	30	30	30	15	15	20	25	25	25
Mannose	15	15	20	20	20	25	15	15	20
Galactose	15	15	25	15	20	20	25	25	25
Mannite	50	70	70	15	30	30	40	60	60
Dulcitol	-	-	10	-	30	50	5	30	40
Lactose	30	40	40	30	40	40	30	40	40
Maltose	10	30	30	15	40	40	15	30	30
Saccharose	-	-	-	5	40	40	20	25	30
Raffinose	-	-	-	15	50	60	10	25	30
Inulin	-	-	5	-	5	5	-	-	-
Dextrin	10	10	15	5	10	10	5	10	10
Starch	-	-	-	-	5	5	-	-	-
Glycogen	-	-	-	-	-	-	-	-	-

It is evident that the fermentation method can not be used as a means of distinction between the *B. colon* and bacillus isolated from the cheese. The latter readily ferments the pentoses, hexoses, di- and trisaccharides. Of the polysaccharides it ferments only dextrin. It was thought, for a time, that its nonfermenting of inulin might be of help in distinguishing it from *B. colon*. Further comparative tests, however, showed that this was not feasible, since the laboratory and feces strains did not always ferment it, and that at times a bubble or two of gas would be formed in tubes inoculated with the bacillus from the cheese.

With the exception of Vaughan and Perkins other investigators did not attempt to study the fermenting properties of their organisms to any great extent. Holst¹⁷ and Kühl¹⁹ merely report that their organisms fermented lactose. Fonteyne¹⁸ found both dextrose and lactose to be nonfermented. Vaughan and Perkins¹⁶ showed that their bacillus fermented dextrose, lactose, saccharose, maltose, dextrin, starch, and glycogen.

The bacillus reduces nitrates to nitrites, but to a smaller degree than the colon bacillus. Using the same strength of nitrate solution the reduction for the Bacillus 3B is two-fifths that for B. colon. It also forms indol.

PATHOGENICITY.

When first isolated the bacillus proved to be pathogenic to mice only. Injections of the original cheese suspension into guinea pigs and rats had no effects. One c.c. of a pure culture of the organism grown in milk killed a mouse, when injected intraperitoneally, in $8\frac{1}{2}$ hours. The organism was recovered from the heart blood, grown in milk, and again injected into a mouse. The mouse died within 10 hours. A rat injected with the same culture (1 c.c.) showed symptoms of being unwell, but did not die. After passage through three more mice the organism had increased in virulence to kill a rat. A guinea pig inoculated with the same culture that killed the rat was unaffected.

The organism was then passed through four rats, and after the last passage was isolated from the heart blood, plated on agar, and obtained in pure culture. It was then grown in milk for 24 hours at 37° C. and 1 c.c. injected, intraperitoneally, into a guinea pig. Death occurred in about 20 hours. The bacillus was then passed through two successive guinea pigs and again isolated in pure culture. Owing to the rush of other work the agar slant was left in the incubator (37° C.) for about 25 days; and at the end of that time it was found that the organism had lost its fermenting power and also its pathogenicity.

It was thought that possibly, owing to its selective media, the bacillus would gain in virulence by repeated transplanting into milk or lactose broth. Such was, indeed, the case. A tube of lactose broth was inoculated with the organism from the stock culture, incubated at 37° C. for 24 hours, and then transplanted into lactose broth. After five successive transplants the organism was found to be again pathogenic to guinea pigs.

That the lactose increased the virulence of the organism seemed evident. Dextrose had a similar effect. A tube each of bouillon, lactose bouillon, milk, and an agar slant were inoculated with the bacillus, incubated at 37° C. for 24 hours, and then 1 c.c. from each of the cultures injected, intraperitoneally, into guinea pigs, the agar slant being taken up in 10 c.c. of physiological salt solution. The pigs injected with the lactose bouillon and milk died in 8 hours; that with the bouillon culture, in 20 hours; while the pig injected with the suspension from the agar slant did not die until 28 days later. The explanation undoubtedly lies in the fact that an anaerobic condition, produced by the presence of sugars, increases the pathogenicity of the organism.

Occasionally some animals seemed to be refractory to seemingly fatal doses. Rats, especially, would not always show the same susceptibility. In order of susceptibility can be placed mice, guinea pigs, rabbits, cats, rats, and dogs.

The bacillus was passed through twenty guinea pigs and then isolated from the heart blood of the last pig. It was found that the virulence of the organism had increased so that .25 c.c. of a 24 hour lactose culture would kill a 200 gm. guinea pig within 20 hours. Intravenous injections did not seem to indicate any greater virulence than intraperitoneal. Subcutaneous injections were also fatal.

POSTMORTEM APPEARANCE OF GUINEA PIGS.

The picture presented by a guinea pig killed with a fatal dose of the organism was usually that of a marked peritonitis. As a rule the lungs were slightly distended, the heart in diastole, and filled with blood. The liver, kidneys, and spleen were normal, although the latter at times was dark and slightly enlarged. The amount of peritoneal fluid present was variable, ranging from none to 5 or even 6 c.c. Usually it was present in an excess from the normal. This fluid was highly toxic, and killed cats which were not killed by lactose broth cultures of the organism. In most guinea pigs there was marked inflammation at the point of injection.

DISTRIBUTION OF THE ORGANISM.

The bacillus is found in the heart blood, lungs, kidneys, liver, spleen, bile, and the peritoneal fluid. In greatest numbers, and in practically pure culture it is found in the peritoneal fluid. In the liver and heart blood it is present in small number. It is numerous in the spleen, kidneys, lungs and bile.

EFFECTS ON CATS.

On September 29, 1916, a cat which had been starved for 24 hours was injected, I.P., with 4.5 c.c. of a 24 hours' lactose broth culture. The cat soon showed signs of unrest, and attempted to vomit. She remained in a comatose condition with high fever, the temperature reaching 41.6° C. 35 hours after injection. She recovered on the second day. The temperature of normal cats was then established previous to a series of tests as to their febrile reactions with the organism. Four cats were used, the temperature taken once daily for four days.

TABLE VI.
NORMAL TEMPERATURE OF CATS.

DATE	TIME	CAT 1	CAT 2	CAT 3	CAT 4
Oct. 4	10.00 A.M.	38.05	38.15	38.65	38.10
5	11.30 A.M.	38.45	37.80	38.40	38.80
6	2.45 P.M.	39.10	38.40	38.80	39.50*
7	10.30 A.M.	38.30	38.45	39.00	37.90
Average Temperature		38.47	38.20	38.71	38.26
Average Temperature of the four cats.....		38.41			

*Not included in the average, as the cat was highly excited.

Several cats were then subjected to injections of the organism and their temperatures taken.

October 9. Cat 4. Weight 3250 gm. This cat was injected, I.P., with 5 c.c. of a 24 hour lactose broth culture, at 9:45 A.M. Retching, vomiting, and purging took place within half an hour.

Temp. at 9.45 A.M. 38.20
10.45 " 39.62
11.45 " 40.12
1.40 P.M. 40.58

Temp. at 2.30 P.M. 41.18
4.30 " 40.50
Oct. 10, 9.30 A.M. 39.10
12.00 " 38.50

The cat recovered.

October 10. Kitten 2. Weight 580 gm., was given, I.P., 3 c.c. of a 48 hour culture (lactose broth) at 9.45 a. m. Retching, vomiting, and purging took place in 35 minutes.

Temp. at 9.45 A.M. 37.12
10.45 " 38.76
11.45 " 40.15
1.15 P.M. 39.78
2.15 " 39.30

Temp. at 3.15 P.M. 39.16
4.15 " 39.20
5.15 " 39.50
8.00 " 38.86
10.00 " 38.44

The kitten recovered.

October 11. Cat 2, weight 2500 gm., was given I.P., 8 c.c. of an 18 hour milk culture of the organism at 9.15 A.M. Retching, vomiting, and purging took place in 30 minutes.

Temp. at 9.15 A.M.	38.12	Oct. 12, at 10.00 A.M.	39.88
10.15 "	38.80	11.15 "	40.10
11.15 "	38.42	1.30 P.M.	39.58
1.00 P.M.	38.72	3.30 "	39.10
3.00 "	38.74	4.45 "	39.62
8.00 "	40.00	Oct. 13 at 9.45 A.M.	38.04
10.00 "	39.40		

The cat refused food for two days. It is interesting to note that with the milk culture a longer sustained fever was obtained than with the lactose broth cultures.

Allusion has already been made to the marked toxic effect of the peritoneal fluid of animals killed by the bacillus. This fluid was of a much higher potency than either the milk or lactose broth cultures.

November 25. Cat 1, which had recovered from a dose of the pure culture given on September 29, was injected, I.P., with 5 c.c. of peritoneal fluid from Rabbit 116, killed by a lactose broth culture of the organism. Retching, vomiting, and purging took place in 20 minutes. The animal was found dead on the morning of the 26th. At the autopsy the heart was found to be in diastole, the blood being fluid. The lungs were slightly distended; the kidneys, hyperemic; the spleen, normal; and the liver, somewhat pale. No fluid was found in the peritoneum, although the viscera were moister than normally. The intestines were pale. The bacillus was recovered from the heart-blood and from the bile, and was shown to be present in smears made from the liver, lungs, spleen, and kidneys.

November 25. Cat 4. This cat was injected, I.P., with 2.5 c.c. of peritoneal fluid from a guinea pig killed by the bacillus. Retching, vomiting, and purging followed within half an hour, the vomiting and purging continuing, intermittently, for about 8 hours. The cat was found dead on the morning of the 27th. The autopsy showed the same picture found in Cat 1. The organism was recovered from the heart blood and bile, and was again shown to be present in the smears made from the liver, lungs, spleen, and kidneys.

December 1. Cat 2 was given, I.P., 3 c.c. of peritoneal fluid from a rabbit killed by the bacillus 3B. Retching, vomiting, and purging took place in 35 minutes.

Temp. at time of injection, 10.00 A.M.	37.00
11.00 "	37.60
12.00 "	37.48
1.15 P.M.	40.20
2.15 "	39.28
3.15 "	39.56
4.15 "	39.20
5.15 "	40.40

Dec. 2, 9.30 A.M. 38.48

The cat died at 5 P.M. on the 2nd. The postmortem findings were similar to those of the preceding cats. The bacillus was shown to be present in the same organs.

December 8. Kitten 1, weight 650 gm., was given, I.P., 2 c.c. of peritoneal fluid from a guinea pig. Retching, vomiting, and purging took place in half an hour.

Temp. at time of injection, 9.40 A.M.	37.32
10.40 "	39.00
11.40 "	39.00
1.30 P.M.	39.90
2.30 "	39.56
3.30 "	39.50
4.30 "	38.80
5.30 "	38.70
9.00 "	35.80

The kitten died late in the night. The postmortem findings were similar to those already described.

It is interesting to note that the temperature rose higher in the cats than in the kittens, although both were equally susceptible to the organism.

To summarize the tests with cats: The symptoms produced were retching, vomiting, and purging, generally within half an hour after injection. The stools were watery and copious. Extreme weakness so that the animal could not voluntarily stand up, and inability to eat food even after a previous starvation of 24 hours were characteristic. A marked fever was produced, the rise in temperature being marked the first few hours after injection. In all cases where the cats were injected with the peritoneal fluid death occurred; where given the lactose broth or milk cultures they recovered. Half-grown cats seemed to be the best adaptable for experimental purposes.

EFFECTS ON RABBITS.

Rabbits proved also susceptible to the organism. As with the cats the peritoneal fluid from animals killed by the bacillus was highly toxic to rabbits. The temperature did not reach the maximum obtained in the cats, although it showed an appreciable rise. In many of the rabbits there was a marked collapse, at times approaching paralysis. As a rule, large doses of the culture were necessary to cause death.

November 24. Rabbit 114 was injected, I.P., at 10.15 A.M., with 4 c.c. of peritoneal fluid from a guinea pig. Death occurred in 4 hours. The heart was in diastole; the blood, fluid. The blood vessels were distended. The organs appeared normal. There was very little fluid in the peritoneum.

November 24. Rabbit 116 was injected, I.P., at 9 P.M., with 4 c.c. of peritoneal fluid from a guinea pig. The rabbit was found dead early the next morning. The autopsy gave the same findings as those of rabbit 114, with the exception that there were about 5 c.c. of a dark fluid in the peritoneum. The bacillus was demonstrated in smears made from the lungs, kidneys, spleen, liver, heart blood, bile, and the peritoneal fluid.

December 1. Rabbit 133 was injected, I.P., with 1.5 c.c. of peritoneal fluid from a guinea pig. Temperature readings were taken at regular intervals.

Temp. at time of injection,	10.00 A.M.	38.35
	11.00 "	37.98
	12.00 "	38.38
	1.15 P.M.	38.72
	2.15 "	38.84
	3.15 "	39.82
	4.15 "	39.92
	5.15 "	39.40

Dec. 2, 9.30 A.M. 38.40

The rabbit died at 3.00 P.M. on December 2. The post mortem findings were similar to those already described.

Intravenous injections proved fatal when 1.5 c.c. of a 24 hour culture (lactose broth) were given.

December 8. Rabbit 160 was injected, I.V., with 1.5 c.c. of a 24 hour lactose broth culture at 10.30 A.M. The rabbit died early in the morning on the following day.

December 8. Rabbit 170 was injected, I.V., with 1.0 c.c. of a 24 hour culture (lactose broth) at 9.30 A.M.

Temp. at	9.30 A.M.	38.35
	10.30 "	39.30
	11.30 "	39.56
	2.00 P.M.	39.96
	3.00 "	39.48
	4.00 "	38.64
	5.00 "	38.90

The rabbit recovered.

EFFECTS ON DOGS.

The effect of feeding dogs with the pure culture of the organism was tried. Finely chopped meat was saturated with a lactose broth culture of the bacillus and given to two dogs, who ate it with apparent relish. No ill-effects were visible.

December 13. A small dog, 3.5 Kg., was given, I.P., at 2 P.M., 5 c.c. of a 24 hour lactose broth culture of the bacillus. No retching nor vomiting, but purging was very marked. The temperature range for the period immediately after injection was as follows:

2.00	P.M.	38.3
3.00	"	39.5
4.30	"	40.1
5.30	"	40.1
7.00	"	39.2
8.00	"	38.8

The dog recovered slightly, but refused food or ate very sparingly. Death occurred early on the morning of December 19. Autopsy: The blood vessels of the mesentery and peritoneum were enlarged. The heart was enlarged and the blood fluid. The vena cava was distended; the veins throughout the body were congested. The gall bladder was distended; the liver was large, with marked hyperstatic congestion. The lungs were normal. The kidneys were enlarged and hyperemic; when cut, they showed many bleeding points. The periphery was of dark color. The bacillus was recovered from the heart blood.

EXPERIMENTS WITH MILK CULTURES.

With the view of determining whether tyrotoxinon was formed by the bacillus, experiments were conducted with milk cultures. The method of procedure was as follows:

Skimmed milk in 1000 c.c. portions was sterilized in the autoclave at 115° C. for 20 minutes. It was then inoculated with a loopful of the organism from a 24 hour agar slant, and incubated at 32° C. to 35° C. for 30 days. At the end of the incubation period the whey was separated from the curd by filtration through paper, and made sterile by filtration through a Berkefeld candle. The whey was then made alkaline with sodium hydrate and extracted with ether.

Vaughan and Perkins found that ether from certain commercial houses, on evaporation, left a residue which, when taken up in water and injected into guinea pigs, caused death. In all the work with ether alkaline extracts, therefore, controls were run on the purity of the ether. In no case was the ether found to contain a toxic residue.

The ether extract obtained from the whey was in the form of a gel, and when evaporated to dryness, left a caramel-colored residue, soluble in water. At no time were crystals obtained, and while the extract showed a slightly poisonous action, no tests made indicated the presence of tyrotoxinon.

The curdling of the milk, as a rule, took place within seven days; but the curd was, even at the end of a month, rather viscous and difficult to separate from the whey. The whey was always strongly acid to litmus. Intraperitoneal injections of the sterile whey into guinea pigs in 5 c.c. and 10 c.c. quantities had no effects. That the organism was not killed by its metabolic products were proved by inoculating bouillon tubes with a loopful of the whey; growth was always obtained, and sugars were fermented.

The alkaline ether extract, when taken up in water, gave a neutral reaction to litmus. In one or two of the portions the reaction was faintly acid. It had usually a rancid, almost putrid odor, and a slightly irritating taste. No positive

Biuret and Millon tests were given, although in one or two of the portions there seemed to be very faint positive reactions. In all of the extracts a strongly positive α naphthol test was given.

The data in Table VII shows that the extract is slightly toxic. The alkaline ether extract was taken up in sterile water so that 10 c.c. represented the liter of the original milk.

TABLE VII.

THE EFFECTS OF ALKALINE ETHER EXTRACTS OF WHEY UPON GUINEA PIGS.

DATE	GUINEA PIG	INJECTION	AMOUNT	RESULT
Dec. 7	1	I.V.	2.0 c.c.	Death in 20 hours
7	2	I.V.	2.0 c.c.	Sick; recovered
7	3	I.V.	5 c.c.	Death in 48 hours
7	4	I.V.	7 c.c.	Death in 22 hours
8	5 to 10 inc.	I.V.	1 c.c.	No effects
8	11	I.P.	10 c.c.	Death in 7 days
12	12 to 14 inc.	I.V.	5 c.c.	Sick; all recovered

Attempts were made to obtain, if possible, the poisonous part in the extract by condensation through alternating freezing and thawing, a process used successfully in this laboratory by Ned R. Smith in his work on water and alcohol organ

TABLE VIII.

PROTECTION ACQUIRED BY GUINEA PIGS THROUGH ETHER EXTRACTS.

Oct. 27	G. P. 1 given	1 c.c. of extract, I.P.	No effects
Nov. 2	" " "	2 c.c. of a 24 hour culture of B. 3B;	death in 12 days
Oct. 27	" 2 "	1 c.c. of extract I.V.	No effects
Nov. 2	" " "	2 c.c. of a 24 hour culture of 3B;	death in 16 days
" 2	Control G. P.	1 c.c. of a 24 hour culture of 3B;	death in 12 hours
Oct. 27	G. P. 3 given	2 c.c. of extract, I.V.	Shock; recovered
" 30	" " "	1 c.c. of 24 hour culture of 3B;	death in 14 days
" 30	Control G. P.	1 c.c. of a 24 hour culture of 3B;	death in 12 hours
Dec. 8	G. P. 4 given	1 c.c. extract, I.V.	No effects
" 12	" " "	0.5 c.c. extract, I.P.	
" 21	" " "	1 c.c. of 24 hour culture of 3B.	No effects
" 8	" 5 "	1 c.c. extract, I.V.	No effects
" 12	" " "	1.25 c.c. extract, I.P.	
" 21	" " "	1 c.c. 24 hour culture 3B.	No effects
" 8	" 6 "	1 c.c. extract, I.V.	No effects
" 12	" " "	1.5 c.c. extract, I.P.	
" 21	" " "	1 c.c. 24 hour culture 3B.	Death in 4 days
" 21	Control G. P. for G. P. 4, 5, 6 given	1 c.c. of a 24 hour culture 3B.	Death in 18 hours
" 12	G. P. 7 given	5 c.c. extract, I.V.	No effects
" 17	" " "	5 c.c. " I.P.	
" 21	" " "	5 c.c. " I.P.	
" 26	" " "	1 c.c. of 24 hour culture 3B.	No effects
" 12	" 8 "	5 c.c. extract, I.V.	No effects
" 17	" " "	5 c.c. " I.P.	
" 21	" " "	5 c.c. " I.P.	
" 26	" " "	1 c.c. of 24 hour culture 3B.	Death in 11 days
" 12	" 9 "	5 c.c. extract, I.P.	No effects
" 17	" " "	5 c.c. extract, I.P.	
" 21	" " "	" " "	
" 26	" " "	1 c.c. of 24 hour culture of 3B.	Death in 20 days
" 26	Control G. P. for G. P. 7, 8, 9, given	1 c.c. of a 24 hour culture of 3B.	Death in 5 hours

extracts. The alkaline ether extract was taken up in sterile water, put into a freezing mixture, allowed to freeze, thawed at room temperature, frozen again, etc., until the solution showed two distinct layers, a colored and a colorless layer. The colored layer was pipetted off, and portions injected into guinea pigs. I.V. injections of the colored portions in 1 c.c. doses had no effects; 2 c.c. killed a guinea pig in 6 days; 2 c.c. of the colorless portion, injected I.P., killed a guinea pig in 4 days. On January 25, four guinea pigs were injected, I.P., with 1 c.c. each of the colored portion. Two died in three days, while the remaining two were unaffected.

A striking feature of the experiment with the alkaline ether extracts was the protection acquired by guinea pigs against fatal doses of the germ after they had been injected with the extract. This is shown in Table VIII.

Of the nine guinea pigs inoculated with the alkaline ether extract three were unaffected by fatal doses of the organism. The remaining six died after an interval ranging from four days to twenty days. The controls succumbed promptly from five to twenty hours. From these results it is evident that a fair degree of protection can be obtained against fatal doses of the organism, a protection which may approach immunity. Milk is not a good medium for the elaboration of the poison, but may become so under favorable conditions. The bacillus does not form tyrotoxicon.

FILTRATES OF LACTOSE BROTH CULTURES.

The marked pathogenicity of the organism when grown in lactose broth and the slightly toxic products obtained in milk cultures led to the study of the toxicity of sterile filtrates from lactose broth cultures. For the first test a broth containing 2% lactose and 5% glycerine was used. The broth, neutral to phenolphthalein, was inoculated with the bacillus, and incubated at 32° C. to 35° C. for 9 days. Several drops of tricresol were then added, and the culture filtered on the following day through a Berkefeld candle. The filtrate was tested for sterility.

On December 21 three guinea pigs were inoculated with increasing amounts: 0.5 c.c., 1 c.c. and 2 c.c. of the sterile filtrate. The pigs were restless for a short time but otherwise showed no ill effects. The filtrate was allowed to stand in a test glass, in a dark place, until January 17, 1917, on account of press of other work. Considerable condensation had taken place. When inoculated in 1 c.c. amounts into guinea pigs, intraperitoneally, it now proved fatal to two out of five of these animals, death occurring in 20 hours. On January 18 six more guinea pigs were inoculated with the following results:

G.P. 1	2 c.c.	I.V.	Death in 27 hours
G.P. 2	1 c.c.	I.V.	Death in 72 hours
G.P. 3	1 c.c.	I.V.	Death in 96 hours
G.P. 4	1 c.c.	I.P.	Death in 24 hours
G.P. 5	1 c.c.	I.P.	Death in 24 hours
G.P. 6	1 c.c.	I.P.	Recovered

The heart blood of each pig was found to be sterile. Marked inflammation of the intestines was characteristic in all cases. The organs were normal.

Tests were next made to determine whether the presence of glycerine was necessary or desirable. Three different broths were inoculated with the organism, incubated at 32° to 35° C. for 7 days, several drops of tricresol added, and the cul-

tures filtered on the following day through Berkefeld candles. The filtrates were then tested for sterility.

Broth A contained 2 per cent lactose.

Broth B contained 5 per cent glycerine.

Broth C contained 5 per cent glycerine, 2 per cent lactose.

TABLE IX.

COMPARATIVE TOXICITY OF LACTOSE, GLYCERINE, AND LACTOSE-GLYCERINE FILTRATES.

DATE	G. P.	AMOUNT INOC. I. P.	FILTRATE	RESULT
Jan. 31	1	1.0 c.c.	A	Death in 4 hours
31	2	1.5 c.c.	A	Death in 7½ hours
31	3	2.0 c.c.	A	Sick; recovered
31	4	1.0 c.c.	C	Sick; recovered
31	5	1.5 c.c.	C	Death in 4 hours
31	6	2.0 c.c.	C	Death in 6 hours
Feb. 1	7	1.0 c.c.	A	Death in 18 hours
1	8	1.5 c.c.	A	Death in 40 hours
1	9	1.0 c.c.	B	Sick; recovered
1	10	1.5 c.c.	B	Sick; recovered
1	11	1.0 c.c.	C	Sick; recovered
1	12	1.5 c.c.	C	Sick; recovered

The most toxic filtrate was obtained from lactose broth. It was then questioned whether the amount of sugar influenced the toxicity. To determine this point lactose broths of various strengths were made up, and the cultures grown and treated as already described.

Broth A contained .25 per cent lactose.

Broth B contained .5 per cent lactose.

Broth C contained 1 per cent lactose.

The guinea pigs used weighed from 200 to 225 grams.

TABLE X.

COMPARATIVE TOXICITY OF LACTOSE BROTHS.

DATE	G. P.	AMOUNT INOC., I. P.	BROTH	RESULT
Feb. 14	1	0.5 c.c.	A	Death in 5 hours
14	2	1.0 c.c.	A	Recovered
14	3	2.0 c.c.	A	Death in 48 hours
14	4	0.25 c.c.	B	Death in 7 hours
14	5	0.25 c.c.	B	Death in 12 hours
14	6	0.5 c.c.	B	Death in 3½ hours
14	7	1.0 c.c.	B	Death in 12 hours
14	8	2.0 c.c.	B	Recovered
14	9	0.5 c.c.	C	Recovered
14	10	1.0 c.c.	C	Recovered
14	11	2.0 c.c.	C	Recovered

Broth B, containing .5 per cent lactose, was most toxic, and this strength was used in the rest of the work with the filtrates.

The minimum lethal dose was determined, using varying quantities of the filtrate. One-tenth c.c. was found to be the smallest amount that would kill a 200-250 gm. guinea pig.

TABLE XI.
DETERMINATION OF THE MINIMUM LETHAL DOSE.

DATE	G.P.	WEIGHT IN GRAMS	INOC. I.P.	RESULT
Mar. 12	1	176	0.1 c.c.	Death in 20 hours
12	2	200	0.1 c.c.	Recovered
12	3	203	0.1 c.c.	Death in 9 hours
12	4	208	0.1 c.c.	Death in 3 hours
12	5	216	0.1 c.c.	Death in 4 hours
12	6	224	0.1 c.c.	Recovered
12	7	197	0.01 c.c.	No effects
12	8	180	0.01 c.c.	No effects
12	9	203	0.01 c.c.	No effects
12	10	220	0.01 c.c.	No effects
12	11	238	0.01 c.c.	No effects
12	12	248	0.01 c.c.	No effects

Peculiarities early noticed regarding the toxin were: (1) Small doses invariably were more fatal than large. (2) Death occurred in a few hours. Thus a guinea pig receiving a dose of 0.1 c.c. would die in from 3 to 24 hours, whereas one receiving 1.0 c.c. or more would, in many cases, recover. The M.L.D. of this toxin can not very well be compared to the M.L.D. of either diphtheria or tetanus toxin, since the lethal period is so short.

HEATED FILTRATES.

The toxicity of the filtrate demonstrated, it remained to determine whether the toxin was destroyed by heat,—in other words, whether the toxin was thermolabile or thermostabile. On March 21 a sterile filtrate was heated in a boiling water bath for ten minutes, cooled, and then injected into guinea pigs, intraperitoneally.

G.P. 1	Given 0.5 c.c.	Death in 8 hours.
G.P. 2	Given 0.5 c.c.	Death in 5 hours.
G.P. 3	Given 0.5 c.c.	Death in 4 hours.
G.P. 4	Given 1.0 c.c.	Death in 4 days.
G.P. 5	Given 1.0 c.c.	Recovered.
G.P. 6	Given 1.0 c.c.	Recovered.

The three pigs which had received 0.5 c.c. died within 8 hours, while only one out of three which had received 1 c.c. died, and that one in four days. Suspicion fell upon the use of the tricresol in the culture. Controls were, therefore, run using amounts even larger than in the cultures; but no ill-effects were ever observed. It was concluded that the toxicity was caused by the bacterial products, and was definitely proved by using filtrates to which tricresol had not been added.

On March 28 a sterile filtrate was divided into three parts: Part A was the normal, untreated filtrate; part B, the filtrate kept in a boiling water bath for 15 minutes; and part C, the filtrate kept in a boiling water bath for 60 minutes. When cooled to room temperature all three portions were injected, I.P., in varying amounts, into guinea pigs. The weights of the guinea pigs were, as in all this work, between 200 and 250 grams.

TABLE XII.
TOXICITY OF NORMAL AND HEATED FILTRATES.

G.P.	INOC., I.P.	FILTRATE	RESULT
1	0.1 c.c.	A	Death in 4½ hours
2	0.25 c.c.	A	Death in 7½ hours
3	0.5 c.c.	A	Death in 5½ hours
4	0.25 c.c.	B	Death in 4½ hours
5	0.5 c.c.	B	Death in 18 hours
6	1.0 c.c.	B	Death in 8½ hours
7	0.25 c.c.	C	Death in 30 hours
8	0.5 c.c.	C	Death in 18 hours
9	1.0 c.c.	C	Death in 4½ hours

TABLE XIII.
TOXICITY OF NORMAL AND HEATED FILTRATES.

G.P.	INOC., I.P.	FILTRATE	RESULT
1	0.1 c.c.	A	Recovered.
2	0.25 c.c.	A	Death in 5 hours
3	0.5 c.c.	A	Death in 5 hours
4	0.5 c.c.	B	Death in 18 hours
5	0.5 c.c.	B	Death in 5 hours
6	1.0 c.c.	B	Death in 4½ hours
7	0.5 c.c.	C	Death in 4 hours
8	0.5 c.c.	C	Death in 6 hours
9	1.0 c.c.	C	Death in 20 hours

As can be seen, the poison was not destroyed by heating, at boiling temperature, for one hour. This was corroborated by another series of tests, carried out as above, on April 10. The only change was made in the reaction of the media, where a -1 (1 per cent alkaline to phenolphthalein) was used instead of the neutral.

The postmortem findings were, as a rule, similar to those observed in the guinea pigs dying from the virulent culture. Marked inflammation of the intestines was characteristic in all animals dying from the filtrate, heated or unheated.

EFFECT OF FILTRATE UPON CATS AND RABBITS.

The sterile filtrate, when injected into cats, gave the same symptoms as the virulent culture, but was without fatal effects. The temperature increased as with the virulent culture.

February 15. Cat 7, weight 2250 gm., was given, I.P., 9 c.c. of a sterile filtrate from a .5 per cent lactose broth culture. Retching, vomiting, and purging took place within 45 minutes. The temperature readings were as follows:

Temp. at time of injection,	8.30 A.M.	37.0
	10.00 "	38.82
	12.00 "	39.64
	2.00 P.M.	39.82
	8.00 "	38.46

The cat recovered.

February 16. Cat 8, weight 2700 gm., was given, I.P., at 8.30 A.M., 9 c.c. of a 0.5 per cent lactose broth filtrate. Retching, vomiting, and purging occurred within 35 minutes.

Temp. at	8.30 A.M.	38.4	1.30 P.M.	40.42
	9.30 "	38.8	2.30 "	40.12
	10.30 "	39.0	3.30 "	40.28
	11.30 "	40.1	4.30 "	39.80
	12.00 "	40.1	5.30 "	39.3

The cat recovered.

A rise in temperature was the only effect produced in a rabbit injected with the filtrate.

February 15. Rabbit 180 was given, I.P., 5 c.c. of a 0.5 per cent lactose broth filtrate, at 8:30 A.M.

Temp. at	8.30	A.M.	38.5	11.45	A.M.	40.0
	9.00	"	40.02	1.45	P.M.	39.4
	9.45	"	39.70	8.00	"	38.08
	10.45	"	39.54			

The rabbit recovered.

FILTRATE FROM USCHINSKY'S SOLUTION.

It was considered that if the toxin had a definite chemical composition its study could be facilitated if it were formed in a synthetic medium. Uschinsky's medium was used. After inoculation with the organism and incubation for 7 days at 37° C., the solution was filtered through a Berkefeld candle and tested for sterility. Guinea pigs, injected with the filtrate, however, were not affected. This was not surprising in view of earlier work with cultures of the organism grown in this solution, when it was found that comparatively large doses were necessary to cause death in guinea pigs.

IDENTITY OF THE SOLUBLE POISON.

Attempts were made to isolate the soluble poison by the precipitation method, as with the toxins of diphtheria and tetanus; none, however, succeeded. The identity of the toxic agent remained, therefore, undetermined.

AGGLUTINATION.

The classification of the bacillus isloated from the poisonous cheese, from the study made thus far, would seem to place it in the B. coli group. The formation of a thermostable poison, however, indicates that the organism is not of the true colon type. To determine, if possible, whether a more accurate classification could be made, agglutination tests were carried out. Rabbits were immunized to B. enteritidis, B. colon, B. paratyphoid A, B. paratyphoid B, and Bacillus 3B, and their sera used in the tests.

TABLE XIV.

AGGLUTINATION OF BACILLUS 3B BY IMMUNE COLON, ENTERITIDIS, PARATYPHOID A, AND PARATYPHOID B SERA.

	1/10	1/20	1/50	1/100	1/200	1/300	1/500	1/1000	1/2000
Immune colon serum									
B. colon	++	++	++	++	++	++	++	++	++
B. 3B	++	++	++	++	++	+	-	-	-
Immune enter. serum									
B. Enteritidis	++	++	++	++	++	++	++	++	+
B. 3B.	++	++	+	-	-	-			
Immune Para. A serum									
B. paratyphoid A	++	++	++	++	++	++	++	++	++
B. 3B	+	±	-	-	-				
Immune para. B serum									
B. paratyphoid B	++	++	++	++	++	++	++	+	-
B. 3B	++	++	+	-	-	-			

TABLE XV.

AGGLUTINATION OF B. COLON, B. ENTERITIDIS, B. PARATYPHOID A, B. PARATYPHOID B., AND B. 3B, BY IMMUNE 3B SERUM.

	1/10	1/20	1/50	1/100	1/200	1/300	1/500	1/1000	1/2000
B. colon.	++	++	++	+	-	-	-	-	-
B. enteritidis	++	+	-	-	-	-	-	-	-
B. paratyphoid A	+	-	-	-	-	-	-	-	-
B. paratyphoid B	++	-	-	-	-	-	-	-	-
B. 3B.	++	++	++	++	++	++	++	++	++

A study of Table XIV shows that the bacillus isolated from the cheese, designated as B.3B, was agglutinated by immune colon serum in a dilution of 1/300; by immune enteritidis serum, in a dilution of 1/50; by immune paratyphoid A serum, in a dilution of 1/10; and by immune paratyphoid B. serum, in a dilution of 1/50. The bacillus, therefore, belongs to the colon group. This is further corroborated by the data of Table XV. Of the four organisms (exclusive of B.3B) tested, B. colon was agglutinated in the highest dilution by the immune 3B serum. That the dilution did not exceed 1/100 was somewhat surprising in view of the fact that immune colon serum agglutinated the 3B bacillus in a 1/300 dilution. The relationship of the organism, as shown by its agglutination, to the enteritidis and partyphoid B. bacilli, may explain its formation of a soluble thermostabile poison.

It is to be regretted that no agglutination test could be carried out with the sera of the patients, none being available. Such tests have been carried out by several investigators. Hughes and Healey,²⁶ in their report of the Aldershot epidemic, stated that a colon bacillus isolated from the liver and kidney from a fatal case gave no reaction with the blood sera from other cases. No reaction was also given by the blood sera from several of the cases with Gärtner's bacillus. Berg²⁸ isolated the B. Paratyphoid B from the intestines and organs from a fatal case of cheese poisoning. This bacillus was agglutinated by the blood serum of the wife of the dead man, herself a victim of cheese poisoning. Fonteyne¹⁸ found that samples of blood taken ten days after the accident did not agglutinate the bacillus he had isolated from the cheese. This bacillus was not agglutinated by sera immune to B. Aertrycke, B. de Bruges, and B. Typhoid. Serum immunized to the cheese bacillus agglutinated B. Aertrycke in 1/100 dilution; B. de Bruges, in 1/10 dilution; and B. Typhoid, in 1/250 dilution.

IMMUNITY.

In the immunization experiments, guinea pigs, on account of their susceptibility and adaptability to this kind of work, were used in preference to other laboratory animals. Immunization was obtained most satisfactorily by vaccine treatment. Agar slant of the organism, of 24 hours' growth, were washed with physiological salt solution, filtered through sterile cotton, heated at 58° C. for 1 hour, tested for sterility, and then standardized. Such a vaccine standardized on December 12 was found to contain 400,000,000 organisms per cubic centimeter. On December 13, six guinea pigs weighing from 200 to 220 grams each, were given, I.P., 5 c.c. of this vaccine. On December 17 a second dose of 1 c.c. was given; while the third dose, 1 c.c., was given on December 21. On December 26 all six pigs were given 1 c.c. of a 24 hour culture of Bacillus 3B. None

of the pigs died. A control guinea pig, injected with the same amount of the organism, died within 5 hours.

On February 4, 8 guinea pigs, weighing from 200 to 225 gm., were injected with .5 c.c. each of vaccine, made as above, and standardized so that 1 c.c.=250,000;000. A second injection of the vaccine, 1 c.c. was given on February 8. One of the pigs died on the following day. The third and final injection was given, in 1.5 c.c. amounts, on February 12. On February 19 all were given 1 c.c. of a 24 hour culture of *Bacillus* 3B. One guinea pig died on the 20th; the rest were unaffected. A control guinea pig, injected with .5 c.c. of the virulent culture, died in 16 hours. These tests show that a high degree of immunity is obtainable by vaccine treatment.

SERUM IMMUNITY.

The serum of guinea pigs immunized by several injections of vaccine, when injected into normal guinea pigs, immunized them to fatal doses of the virulent culture.

March 3. Two immune pigs were bled and their sera obtained aseptically. Four guinea pigs (210-225 gm.) were injected, I.P., with 0.1 c.c. of the immune serum. On March 8 one of the pigs was injected with 1 c.c. of a 24 hour lactose broth culture of B.3B. Death occurred 3 days later. A control pig died in 12 hours. On March 12, the second and third pigs were injected with 1 c.c. and 1.5 c.c., respectively, of a 24 hour lactose broth culture of B.3B. Guinea pig 3 died on the sixth day, while guinea pig 2 was unaffected. A control died in 12 hours.

Two guinea pigs injected, March 3, with .25 c.c. of the immune serum, were injected, on March 12, with 1 c.c. of a virulent culture of *Bacillus* 3B. Both were unaffected. A control died in 9 hours.

IMMUNITY TO SOLUBLE POISON.

While it was comparatively easy to obtain immunity to the virulent organism, it was very difficult to obtain immunity to the soluble poison it elaborated. Invariably the guinea pigs would die after the second or third inoculation, regardless of whether these doses were increased or decreased.

Thus, for example:

On March 12, three guinea pigs (200 gm.) were inoculated with .01 c.c. of a sterile lactose broth filtrate. On March 16 each received a dose of .1 c.c. of the filtrate. One died on the second, one on the third, and one on the fourth day after the inoculation. Again, on March 12, three guinea pigs were inoculated with .001 c.c. of the filtrate. On March 16 a second injection of .01 c.c. was given. One pig died on the 20th. The two remaining pigs were inoculated on the 24th, with .5 c.c. of the filtrate. These, as well as a control, died in 4 hours.

On March 21, six guinea pigs (200-250 gm.) were injected with .01 c.c. of the filtrate. On March 26, a second injection, .005 c.c. was given. One pig died on the following day, and one on the 29th. A third injection of .02 c.c. was given on April 7. One pig died on April 8. On April 14 the three remaining pigs and a control were injected with .25 c.c. of a freshly prepared filtrate. One of the pigs and the control died, the former in two days and the latter in seven hours. The other two guinea pigs were unaffected.

Guinea pigs immunized to fatal doses of the poison are immune to fatal doses of the virulent organism. Injections of heated sterile filtrate will immunize guinea pigs to fatal doses of the organism.

Two guinea pigs were injected, I.P., on March 3 with .5 c.c. of a sterile .25 per cent lactose broth filtrate. On March 12 they were given 2 c.c. each of .5 per cent lactose broth filtrate. The third dose, .2 c.c. of a .5 per cent filtrate, they were given on the 16th. On March 24 they were both injected with a freshly prepared .5 per cent filtrate, each receiving

.5 c.c. They were unaffected. A control died in 4 hours. On March 30 both were given 1 c.c. of a 24 hour culture of *Bacillus* 3B. They were unaffected, while a control, given the same dose, died in 15 hours.

On March 28 a .5 per cent lactose broth filtrate was heated, for one hour, in a boiling water bath. Varying quantities were injected into guinea pigs.

- G.P. 1, weight 300 gm., received .25 c.c. No effects.
- G.P. 2, weight 313 gm., received .50 c.c. No effects.
- G.P. 3, weight 330 gm., received 1.0 c.c. Sick; recovered.
- G.P. 4, weight 313 gm., received 1.5 c.c. Sick; recovered.

On April 8 all four received .5 c.c. of the heated filtrate. No effects were observed in any of the animals. On April 17, they were injected, in varying doses, with a 24 hour lactose culture of *Bacillus* 3B.

- G.P. 1 received 2 c.c. of the culture.
- G.P. 2 received 1.25 c.c. of the culture.
- G.P. 3 received 1.0 c.c. of the culture.
- G.P. 4 received 1.0 c.c. of the culture.

All were sick, but recovered on the following day. A 350 gm. control pig, given 1 c.c. of the culture, died in 10 hours.

From the data given it is evident that immunity can be obtained by injections of the sterile filtrate not only to the soluble poison itself, but also to the virulent culture of the organism. The soluble poison is undoubtedly not a true toxin, but evidence has been given that it does form immune antibodies.

CONCLUSIONS.

The data and results obtained in this work warrant the following conclusions.

1. Six cases of cheese poisoning were caused by a toxicogenic bacillus.
2. Morphological and biochemical reactions, and agglutination tests prove that the bacillus belongs to the colon group.
3. That it is not a true colon bacillus is shown by its formation of a soluble thermostabile poison.
4. Poisoning can not be induced in animals either by feeding the poisonous cheese or by feeding with cultures of the isolated organism.
5. Mice, guinea pigs, rabbits, cats, rats, and dogs are susceptible, in the order named, to the bacillus.
6. The bacillus does not form tyrotoxin.
7. Five-tenths per cent lactose broth, neutral to phenolphthalein, is the medium most suitable for the production of the poison.
8. The soluble thermostabile poison produced by the bacillus is a toxin-like body, of unknown chemical constitution.
9. Immunity to the organism can be acquired by vaccine and immune serum injections. It can also be obtained by injections of the sterile, cell-free filtrate from lactose broth cultures.
10. A lesser but yet marked protection can be acquired by injections of alkaline ether extracts of whey from milk cultures.

ACKNOWLEDGMENT.

The writer acknowledges his thanks to Dr. V. C. Vaughan for his kind interest in the work and for his valuable suggestions; also to Dr. H. W. Emerson for aid in several of the postmortems.

BIBLIOGRAPHY.

- ¹Husemann: Handbuch der Toxikologie, 1862, Berlin, 333.
- ²Christison: A Treatise on Poisons, Philadelphia, 1845, 494.
- ³Morgagni: In Orfila: Toxikologie, Berlin, 1819, iv, 316.
- ⁴Hunnefeld: Die Chemische Ausmittelung des Käsegifts, Horn's Arch., 1827, i, 203. Cited by Christison.
- ⁵Vaughan and Novy: Ptomaines and Leucomaines, Phila., 1896, 83.
- ⁶Vaughan: Ein Ptomain aus giftigem Käse, Ztschr. f. physiol. Chem., 1886, x, 146.
- ⁷Wallace: Cases of Cheese Poisoning, Med. News, London, li, 1887, p. 69.
- ⁸Wesener and Rossman: Tyrotoxin in Cheese, Philadelphia Med. Jour., 1898, i, 1119.
- ⁹Newman: In Swithinbank and Newman: Bacteriology of Milk, London, 1903, 208.
- ¹⁰Lepierre: Comp. Rendus 118, 476, 618, 1894. Cited by Vaughan and Novy: Ptomaines and Leucomaines.
- ¹¹Dokkum: Die giftigen Bestandteile des in Fäulnis übergegangenen Käses, Milchzeitung, 1895, 73.
- ¹²Spica: Investigation of Some Cases of Cheese Poisoning, Abstr. Chem. Abstr., 1916, x, 1559.
- ¹³Vaughan and Novy: Ibid., 16.
- ¹⁴Sternberg: Poisonous Cheese, Report Michigan State Board of Health, 1885.
- ¹⁵Vaughan and Novy: Ibid., 95.
- ¹⁶Vaughan and Perkins: Ein in Eiscrème und Käse gefundener gift producirender Bacillus, Arch. f. Hyg., 1896, xxvii, 308.
- ¹⁷Holst: Beobachtungen über Käsevergiftungen, Centralbl. f. Bakteriologie, 1896, xx, 160.
- ¹⁸Fonteyne: Intoxications par le fromage, Presse méd., 1909, xvii, 673.
- ¹⁹Kühl: Über eine Käsevergiftung, verursacht durch eine mit Bacterium lactis aerogenes Escherich übereinstimmende Bacterie, Ztschr. f. Untersuch. d. Nahrungs-u. Genussmittel., 1913, xxv, 193.
- ²⁰Grimm: Vergiftung durch Käse, Schmidt's Jahrbücher, 1837, xv, 17.
- ²¹Rosendahl: Fälle von Vergiftung durch Käse, Schmidt's Jahrbücher, 1839, xxi, 162.
- ²²Zenker: Vergiftung durch Käse, Schmidt's Jahrbücher, 1851, lxix, 170.
- ²³Pollius: Vergiftung mehrerer Personen durch Handkäse, Schmidt's Jahrbücher, 1842, xxxiv, 155.
- ²⁴Report of Michigan State Board of Health, 1885.
- ²⁵Ehrhardt: Zur Casuistik der Vergiftungen durch Käse, Schmidt's Jahrbücher, 1887, ccxiii, 248.
- ²⁶Hughes and Healey: An Acute Epidemic of Gastroenteritis Attributed to Food Poisoning, Lancet, London, 1899, ii, 1223.
- ²⁷Lochte: Die Milch und ihre Bedeutung für Volkswirtschaft und Volksgesundheit, Hamburg, 1903, p. 345.
- ²⁸Berg: Cited by Uhlenhuth and Hübener in Kolle und Wassermann: Handbuch der Pathogenen Mikroorganismen, 1913, iii, 1031.
- ²⁹Cameron: Severe Illness Caused by Ingestion of Cheese, Dublin Jour. Med. Sc., 1882, lxxiii, 116.
- ³⁰Holm: Schmidt's Jahrbücher, 1880, clxxxv, 22.
- ³¹Nüchel: Schmidt's Jahrbücher, 1837, xv, 17.
- ³²Vaughan and Novy: Ibid., 85.
- ³³Bley: Chemische Ausmittelung von Käsegift, Schmidt's Jahrbücher, 1838, xvii, 7.
- ³⁴Kobert: Beiträge zur Kenntniss der Ptomaine, Schmidt's Jahrbücher, 1880, clxxxvi, 123.
- ³⁵Report of Michigan State Board of Health, 1884.
- ³⁶Ibid., 1886.
- ³⁷Wolff: The Ptomaines—Their Forensic and Pathological Importance, Therap. Gaz., 1888, xii, 21.
- ³⁸Reed: Cheese Poisoning Cases at Mansfield, Ohio, Jour. Am. Med. Assn., 1893, xxi, 354.
- ³⁹Vaughan: Tyrotoxin-Cheese Poison, Med. News, London, 1885, xlvii, 111.
- ⁴⁰Husemann: Die Ptomaine und ihre Bedeutung für die gerichtliche Chemie und Toxikologie, Arch. d. Pharm., 1880, ccxiv, 169.
- ⁴¹Uhlenhuth and Hübener: In Kolle und Wassermann: Handbuch der Pathogenen Mikroorganismen, 1913, iii, 1031.
- ⁴²Taylor: On Poisons in Relation to Medical Jurisprudence and Medicine, Philadelphia, 1875, 514.
- ⁴³Pharm. Jour., 1862, iv, 89.
- ⁴⁴Britton: Symptoms of Poisoning Following the Ingestion of Cheese, Lancet, London, 1873, i, 328.
- ⁴⁵Lancet, London, 1873, i, 259.
- ⁴⁶Cairns: Edinburgh Med. Jour., x, No. 2, 1865, 854.

